PRIMARY RESEARCH PAPER | *Philippine Journal of Systematic Biology*
DOI 10.26757/pjsb2019a13008

Siderophores of fungi from Lipa clay loam soil, Philippines

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Abstract

This research was conducted to isolate culturable soil-borne fungi from Lipa clay loam soil and identify the types of siderophores they produce. The frequency of fungal isolates was likewise compared across the soil strata. Soil samples were plated on Rose Bengal Agar (RBA) and incubated for five days. Morpho-culturally unique isolates were transferred in Potato Dextrose Agar (PDA) slants and identified using taxonomic keys and by ITS sequencing. Siderophores of each species were then characterized through colorimetric methods. Twenty-nine species of fungi were identified. Six isolates were Basidiomycetes, 1 Zygomycetes and 22 Ascomycetes. Among ascomycetes, two were teleomorphs: *Chaetomium globosum* Kunze and *Emericella nidulans* (Eidam) Vuillemin. Isolates were then characterized in terms of their ability to produce siderophores. Majority produced hydroxamate type of siderophores while only *Aspergillus tamarii* Kita produced all three types of siderophores. Six other species failed to produce any of the three types. Isolates may further be studied for their metal remediating capacity and other biological activities.

Keywords: *Chaetomium globosum*, *Emericella nidulans*, Lipa clay loam soil, siderophores, soil-borne fungi

Introduction

Many fungi inhabit the soil and perform multiple functions. Beneficial fungi include decomposers, mutualists and antagonists of pathogenic species. Pathogenic ones are either soil-borne or overwinter in the soil during their saprogenic stages. It is estimated that majority of the fungi named are residents of the soil (Bridge and Spooner, 2001). Because of the differential characteristics of soils, fungal composition greatly varies among soil ecosystems. Unique soil-borne fungi can be identified, and their potential applications may be harnessed. One soil type needing fungal characterization is the Lipa clay loam soil.

The Lipa soil series are characteristically undulating to rolling and provides moderately well drainage capabilities. Soils in this series are classified as fine, clayey, mixed, shallow, isohyperthermic Typic Eutrudepts and are residual soils of volcanic tuff. The surface soil, 25–30 cm in depth is dark brown, brown to light brown in color, loose, fine loam. The texture ranges from loam to clayey loam or silty clay loam. In older landscapes, the subsurface horizon is mainly clayey and has medium, angular blocky structure, and sticky and plastic consistency (Carating et al., 2014).

One characteristic of fungal isolates this research wanted to elucidate is their ability to produce chelating substances collectively known as siderophores. Traditionally, siderophores are known only by being iron chelators. Ferric hydroxide has extreme insolubility which limits free iron in aerobic environment. This biological need to access iron is important for microorganisms in the soil to proceed with metabolic processes such as reduction of oxygen for ATP synthesis, reduction of ribotide precursors of DNA, for formation of heme, among many other functions. Similarly, excessive iron can also be toxic because of its catalytic role in the formation of oxidizing radicals from superoxide and peroxide (Loper and Buyer, 1991). As a response, fungi can either release high-affinity ferric iron reductase or synthesize siderophores. Microorganisms have evolved siderophores as a biochemical means to tap on the rich iron deposit of the soil, regulating the amount they incorporate and transporting them into the cell.

Siderophore identification is an important step towards identification of fungi capable of metal remediation. Thus, this research aimed to isolate culturable fungi from Lipa clay loam soil and characterize each isolate in terms of their morpho-

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Date Submitted: 03 May 2019
Date Accepted: 13 March 2020

cultural characteristics and the corresponding siderophores they produced.

Materials and Methods

Sampling of Lipa Clay Loam Soil

Soil used in this research was collected from the University of the Philippines at Los Baños (UPLB) grounds in the following coordinates and elevation: N 14º 08' 44.1" to 14º 08' 45.1" and E 121º 15' 42.4" to E 121º 15' 43.5", 29.8 to 33.6 masl. The soil in UPLB belongs to the Lipa soil series and shows characteristics of clay loam soil. Specifically, the sampling site was at the lower campus near the Institute of Plant Breeding, characterized by patches of grass vegetation and few ornamental plants. A soil auger was used to take a soil core. Each soil core was carefully separated based on the following depths: 0-5 cm, 6-10 cm, 11-20 cm, and 21-30 cm. Approximately 25 kg of soil from each soil depth was taken. All samples from the same soil depth were combined to form one composite sample for use in the isolation of fungi. A sub-sample of the soil was subjected to soil analysis for determination of the following edaphic characteristics: pH, soil moisture, clay, silt and sand composition, and available nitrogen (N), phosphorus (P) and potassium (K).

Isolation and Identification of Soil Mycoflora

Four replicates of soil with 25 g each were processed for isolation of soil mycoflora from each of the collection sites. Twenty-five (25) grams of soil was diluted in 100 mL of sterile distilled water to form the stock solution. Aliquot (1.0 mL) of the stock solution was transferred to 9.0 mL of sterile distilled water to form a 10-fold dilution (10^{-1}). A serial transfer was done up to a 100,000-fold dilution (10^{-5}). One milliliter each of dilution was plated on freshly prepared Rose Bengal Agar (RBA), amended with streptomycin, using spread plate technique. First to the fifth dilutions were plated in triplicate and incubated for 5 d at room temperature. Colonies showing various characteristics based on morphological and visual cultural assessment were transferred into fresh potato dextrose agar (PDA) slants and assigned a morphospecies code.

Isolates were morphologically and culturally characterized to aid in identification through light microscopy and scanning electron microscopy. Agar block technique was utilized to grow fungi and document the formation of fruiting structures. Identification of isolates was aided by morphological keys, primarily that of Watanabe (2010) on soil and seed fungi, and Samuels and Hebbar (2015) on *Trichoderma* species. All isolates were deposited at the UPLB Museum of Natural History Microbial Culture Collection and were assigned unique deposit codes (Table 1).

Likewise, fungal DNAs were extracted from the cultures using the Zymo Quick-DNA^{TM} fungal Miniprep Kit (Catalog No. D6005) following manufacturer's instructions. Extracted DNAs were amplified using the primers ITS 1 5' (TCC GTA GGT GAA CCT GCG G) 3' and ITS 4 5' (TCC TCC GCT TAT TGA TAT GC) 3' and sent to Macrogen Korea for ITS sequencing. The resulting nucleotide sequences were cleared of noises and aligned using ChromasPro and Mega7 softwares. Identities of the isolates were determined by homology search from the National Center for Biotechnology Information (NCBI) database using Basic Local Alignment Search Tool (BLAST). Those with 97% or higher homology percentage were accepted. Fungal diversity was determined using the software Biodiversity Pro (McAleece et al., 1997).

Chemical Assays for Detection of Specific Types of Siderophores

Detection of specific types of siderophores followed the methods described by Payne (1994), Dave and Dube (2000) and Shenker et al. (1992). Cultures were grown in 10-mL Potato Dextrose Broth (PDB) (Kumari and Kaviyarasan, 2014; VanderMolen et al., 2012) for 21 d at 25-30°C (room temperature) and centrifuged at 10,000 rpm for 15–20 min. Cell-free supernatants were used for the chemical tests.

Detection of Hydroxamate Siderophores (Tetrazolium Test)

One to two drops of 2N NaOH and 0.1 mL of test sample were added to a pinch of tetrazolium salt. Formation of a deep red color indicated the presence of hydroxamate siderophore.

Detection of Catecholate Siderophores

Freshly prepared 2% aqueous ferric chloride ($FeCl_3$) (1.0–5.0 mL) was added to 1.0 mL of the test sample. Formation of red wine color and maximum absorbance at 495 nm indicated presence of catecholate siderophores.

Detection of Carboxylate Siderophores (Vogel Test)

Three drops of 2N sodium hydroxide (NaOH) were mixed with 1 drop of phenolpthalein. Water was added until light pink color developed. Disappearance of the color upon addition of 1 mL sample indicated the presence of carboxylate siderophores.

Results and Discussion

Characterization of Lipa Clay Loam Soil Samples

Analysis of Lipa clay loam soil showed the following composition: 39.62% silt, 33.16% clay, and 27.23% sand. Organic matter content was at 3.6% with a pH of 5.4. Available

Table 1. Deposit and accession numbers of fungal isolates at the Microbial Culture Collection, Museum of Natural History, University of the Philippines Los Baños, Philippines and the National Center for Biotechnology Information Genbank.

Isolation code[1]	*Identity*	*Deposit Code*[2]	*Accession Number*[3]
JJGG-71	*Aspergillus brasiliensis*	*MCC-MNH 2514*	MK644021
JJGG-73	*Aspergillus versicolor*	*MCC-MNH 2515*	MK644120
JJGG-54	*Aspergillus terreus*	*MCC-MNH 2516*	MK644096
JJGG-66	*Aspergillus aculeatus*	*MCC-MNH 2517*	-
JJGG-37	*Acrophialaphora levis*	*MCC-MNH 2518*	MK646060
JJGG-40	*Aspergillus flaviceps*	*MCC-MNH 2519*	MK645221
JJGG-26	*Aspergillus flavus*	*MCC-MNH 2520*	MK645222
JJGG-63	*Aspergillus fumigatus*	*MCC-MNH 2521*	MK644062
JJGG-64	*Aspergillus tamarii*	*MCC-MNH 2522*	-
JJGG-65	*Aspergillus tubingensis*	*MCC-MNH 2523*	MK644176
JJGG-38	*Penicillium pinophilum*	*MCC-MNH 2524*	MK646032
JJGG-30	*Penicillium polonicum*	*MCC-MNH 2525*	MK64603
JJGG-55	*Penicillium solitum*	*MCC-MNH 2526*	MK567887
JJGG-51	*Penicillium citrinum*	*MCC-MNH 2527*	-
JJGG-36	*Talaromyces radicus*	*MCC-MNH 2528*	MK646059
JJGG-67	*Talaromyces pinophilus*	*MCC-MNH 2529*	MK646041
JJGG-41	*Rigidoporus vinctus*	*MCC-MNH 2530*	MK646062
JJGG-50	*Schizophyllum commune*	*MCC-MNH 2531*	MK680083
JJGG-74	*Emericella nidulans*	*MCC-MNH 2532*	MK646070
JJGG-52	*Trichoderma erinaceum*	*MCC-MNH 2533*	-
JJGG-45	*Trichoderma harzianum*	*MCC-MNH 2534*	MK646064
JJGG-53	*Fusarium sublunatum*	*MCC-MNH 2535*	-
JJGG-33	*Chaetomium globosum*	*MCC-MNH 2510*	-
JJGG-46	*Mortierella* sp.	*MCC-MNH 2536*	-
JJGG-44	*Trametes maxima*	*MCC-MNH 2537*	MK646061
JJGG-49	*Fomitopsis meliae*	*MCC-MNH 2538*	MK646068
JJGG-32	*Emmia lacerata*	*MCC-MNH 2539*	-
JJGG-42	*Talaroymces verruculosus*	*MCC-MNH 2540*	-
JJGG-43	*Lentinus sp.*	*MCC-MNH 2541*	MK646067

[1]unique strain identifier
[2]deposit number at the UPLB-Museum of Natural History
[3]accession number at the National Center for Biotechnology Information

P was 11 ppm, K at 2.05 me/100g of soil, while total N was 0.19%. Soil CEC was 40.06 me/100g of soil. Zinc and Cu were also analyzed to provide baseline for initial concentration in the soil. Zn was found to be at 2 ppm while Cu was at 9 ppm.

Identification andCharacterization of Fungal Isolates

A total of 29 species were identified by morphological and molecular techniques (Table 1). Amplification of all fungi using ITS1 and ITS4 primers yielded fragment length of 580-750 bp, all falling within the expected range for the primers used. Basidiomycetes accounted for 20.67% (6 out of 29 species) of isolates. Among Ascomycetes, only 2 were teleomorphic. These were *Chaetomium globosum* Kunze and *Emericella nidulans* (Eidam) Vuillemin that form perithecium and cleistothecium, respectively. These coelomycetes were photomicrographed under the scanning electron microscope to observe their ascoma and spores. Of the remaining ascomycetes, 9 were *Aspergillus*, 4 were *Penicillium,* 3 *Talaromyces*, and 2 *Trichoderma* species. Representatives of each group are presented for reference in the accompanying figures (1-8). The rest of the isolates belonged to individual genera. One species, *Morteriella* sp., belonged to Zygomycota. The hyphomycetes were members of only three families: Trichocomaceae, Nectriaceae, and Hypocreaceae. They are common soil inhabitants, mostly with a saprophytic

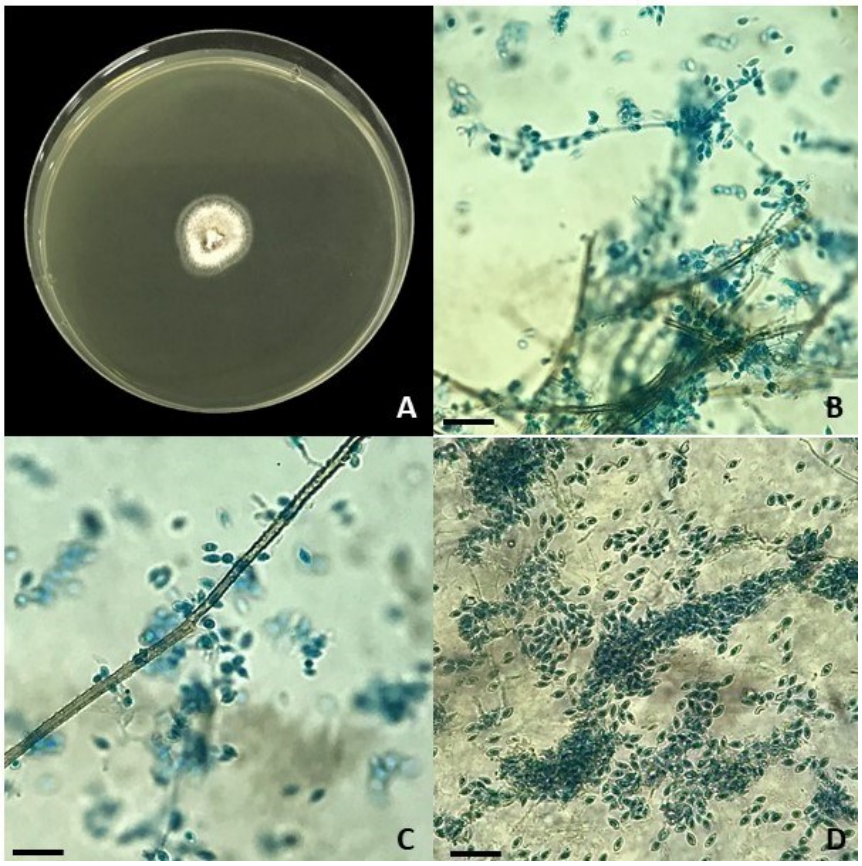

Figure 1. Seven-day-old *A. levis* on PDA (A), hyaline fusiform ascospores and ornamented mycelia (B-D). Scale bar = 20 µm.

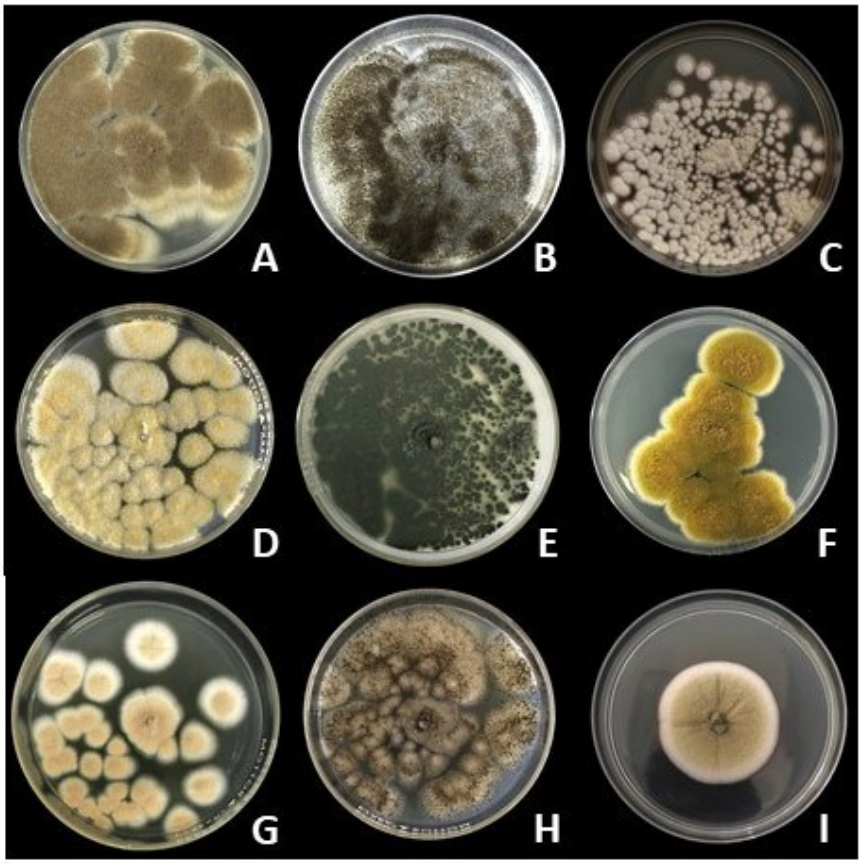

Figure 2. Seven-day-old *Aspergillus* species on PDA: *A. aculeatus* (A), *A. brasiliensis* (B), *A. flaviceps* (C), *A. flavus* (D), *A. fumigatus* (E), *A. tamarii* (F), *A. terreus* (G), *A. tubingensis* (H) and *A. versicolor* (I).

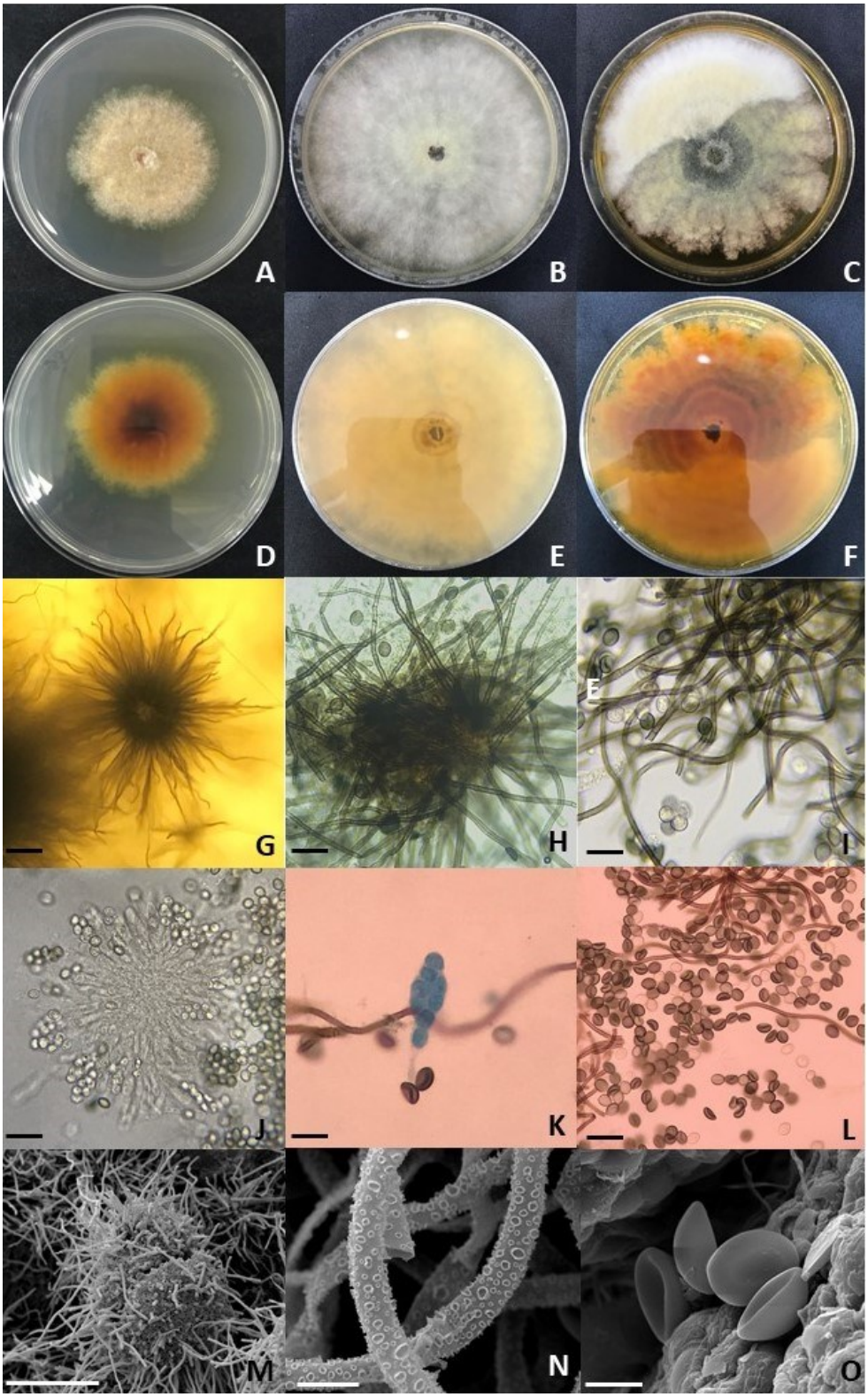

Figure 3. Seven-day-old *C. globosum* on PDA (A, D), yeast extract agar (YEA) (B,E), malt extract agar MEA (C,F), perithecium (G,H), ascomatal hairs (I), fasciculate asci (J), lactophenol-stained ascus with eight ascospores (K) and mature ascopores (L); scanning electron micrograph ofperithecium (M), ascomatal hairs (N) and ascospores (O). Scale bars: G-L = 10 µm; M = 100 µm; N,O = 5 µm.

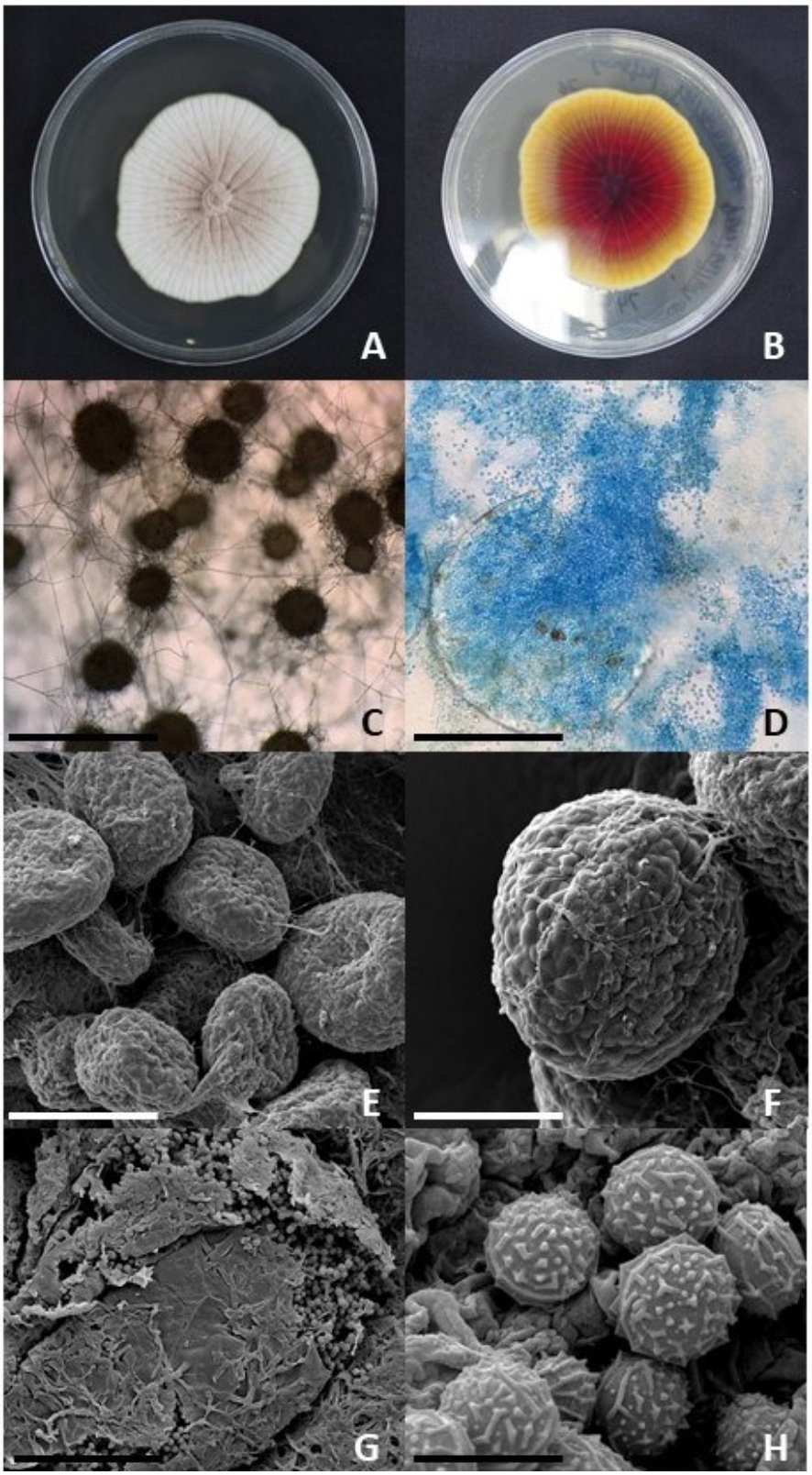

Figure 4. *E. nidulans* on PDA seven days after inoculation (A) and reverse side (B); intact (C) and ruptured cleistothecium with globose ascospores (D); scanning electron micrograph of cluster of cleistothecia (E) and single cleistothecium (F) showing roughened ascoma wall; ruptured cleistothecium (G); ascospores (H). Scale bars: C = 300 µm; E = 100 µm; D, F, G = 50 µm; H = 3 µm.

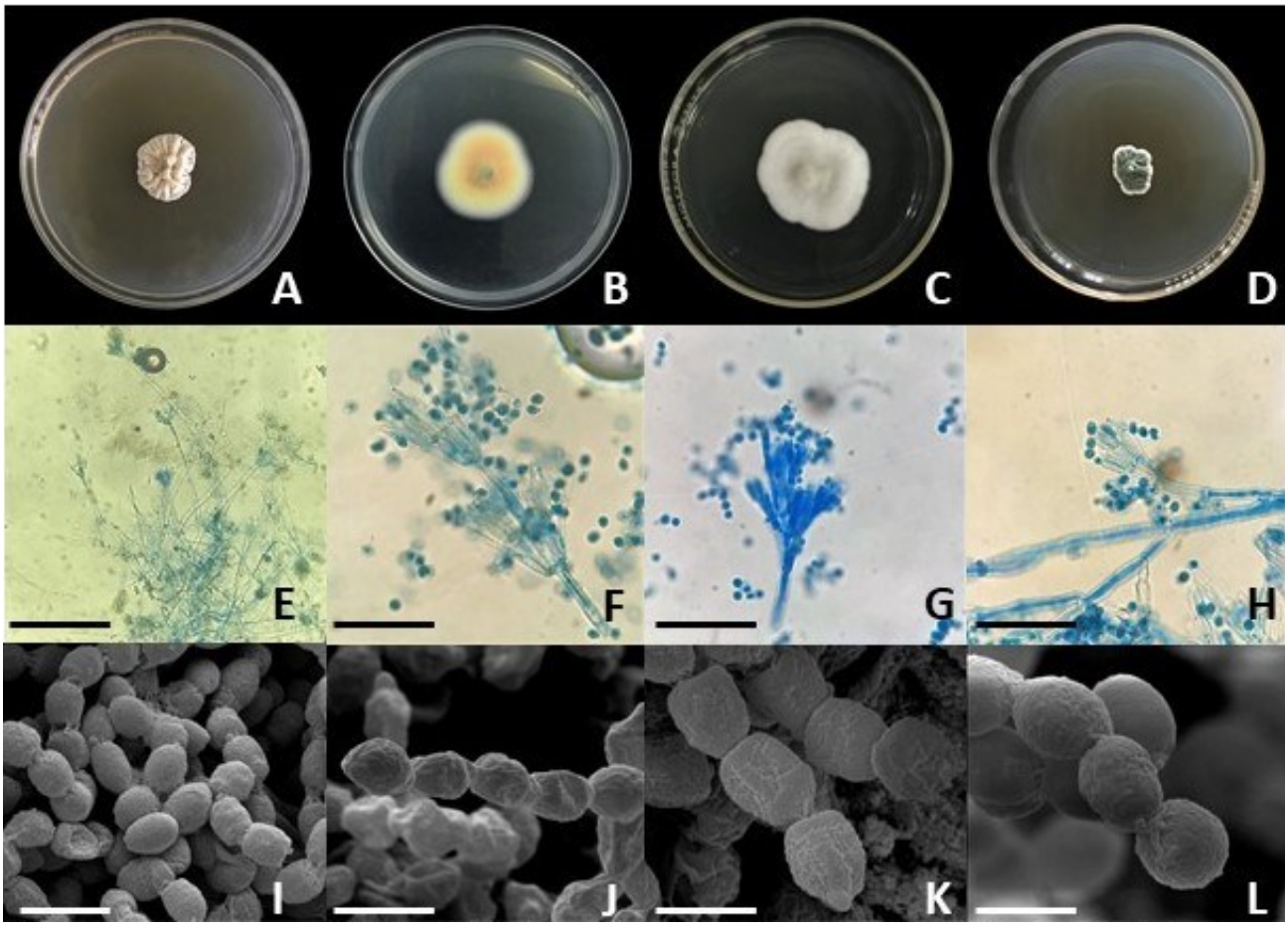

Figure 5. Seven-day-old *Penicillium* species on PDA: *P.citrinum* (A), *P. pinophilum* (B), *P. polonicum* (C) and *P. solitum* (D); micrographs of phialides and catenate conidia of : *P.citrinum* (E), *P. pinophilum* (F), *P. polonicum* (G) and *P. solitum* (H); scanning electron micrographs of conidia of : *P.citrinum* (I), *P. pinophilum* (J), *P. polonicum* (K) and *P. solitum* (L). Scale bars: E-H = 30 µm; I = 5µm; J-L = 3 µm.

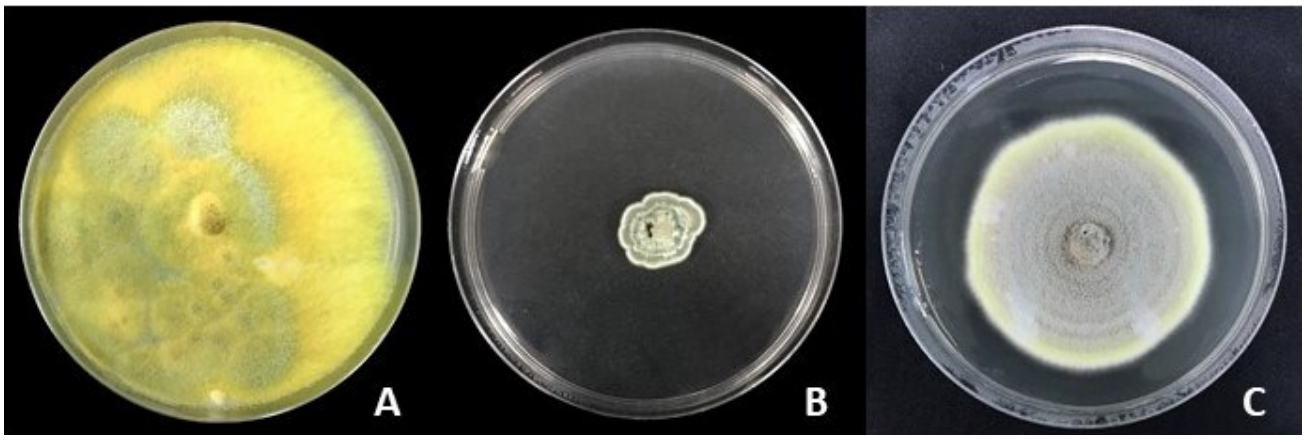

Figure 6. Seven-day-old *Talaromyces* species on PDA: *T. verruculosus* (A), *T. radicus* (B) and *T. pinophilus* (C).

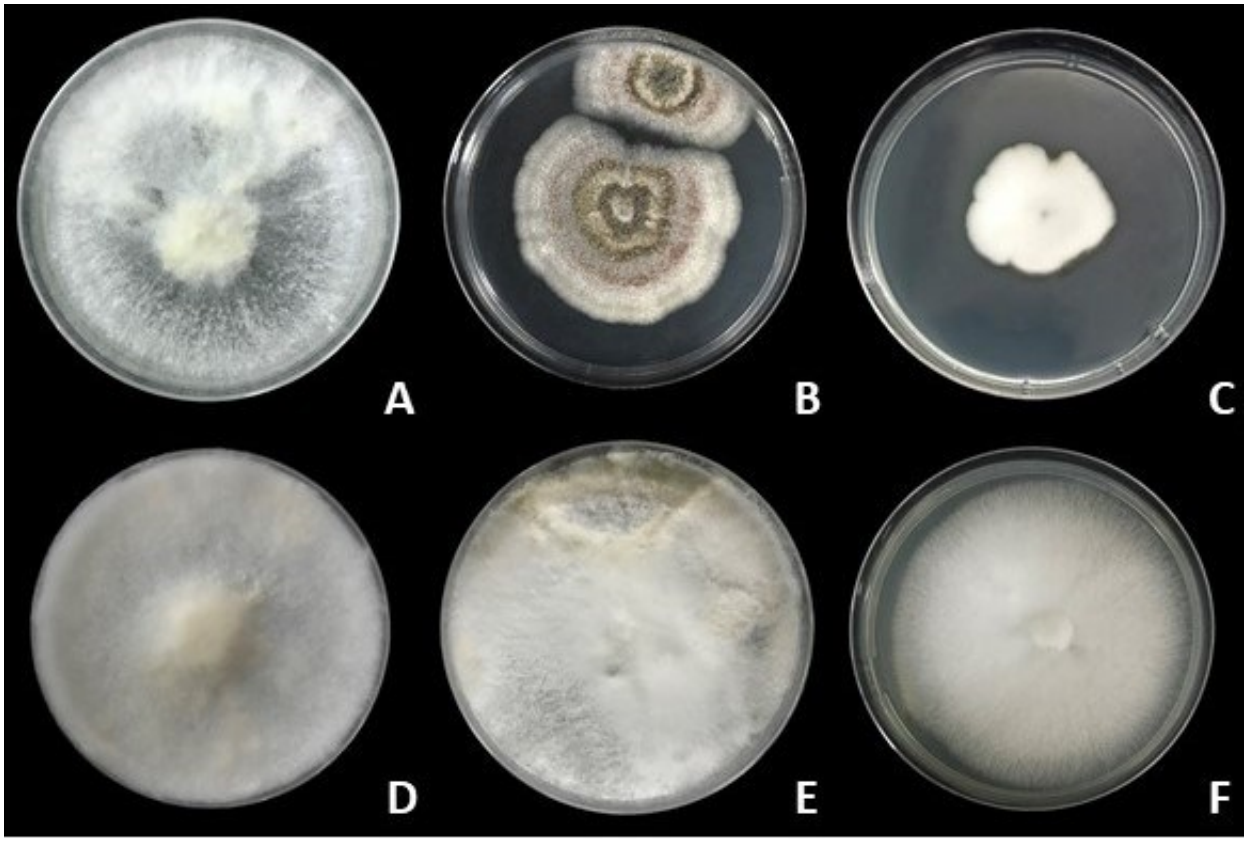

Figure 7. Basidiomycetes growing on PDA: *Emmia lacerata* (A), *Fomitopsis meliae* (B), *Lentinus* sp. (C), *Rigidoporus vinctus* (D), *Schizophyllum commune* (E), and *Trametes maxima* (F).

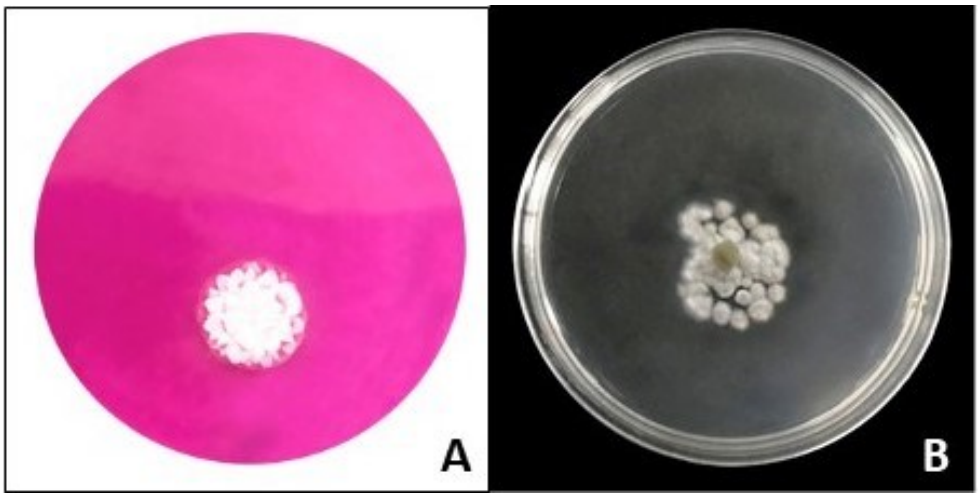

Figure 8. *Mortierella* sp. growing on RBA (A) and PDA (B).

role.

Fungal Colony-forming Units (CFU) at Different Sampling Depth

Colony-forming units represent a rough approximate of the number of microorganisms in the soil sample. These CFUs, as shown in Table 2, decreased drastically as the depth of soil increased. This is to be expected as fungi are aerobic organisms and would thrive at soil depth with adequate oxygen levels. The surface soil at 0–5 cm depth is the area most exposed to air and is constantly subjected to soil movement and aeration. Statistical analysis showed that this specific depth has significantly higher number of soil fungi than at other depths ($p = 0.0008$). The number of unique morphotypes also decreased, suggesting that there are fewer culturable taxa as soil sampling gets deeper. Table 2 shows that the number of morphotypes differs in number of unique species. During isolation, morphologically different fungi growing on plate were isolated and assigned an isolation code. At final identification, some of these were combined because they belong to the same species after thorough morphological and molecular analysis. Frequency of each unique species are then summarized in Table 3. Unique species refer to isolates that are morphologically and molecularly distinct after analysis.

As expected, the *Aspergillus* and *Penicillium* species, including *Talaromyces*, were ubiquitous in the soil strata, and occurred in all layers of the soil. These 3 genera are known for their gregarious sporulation. All Basidiomycetes, on the other hand, were not found in the deeper part of the soil. In relation to the grass cover of the sampling sites, Basidiomycetes may fall into four functional groups (Griffith and Roderick, 2008): dung fungi, litter decomposers, terricolous species and root endophytes. All these variables are found in the upper layer of the soil.

No strict plant pathogen was isolated in this study. *Acrophialophora levis* Samson & T. Mahmood, on one hand, is reported to be an opportunistic human pathogen which can cause keratitis, pulmonary colonization and other serious infections (Sandoval-Denis et al., 2015).

The overall diversity is 1.07, qualitatively described to be low. The diversity indices for each soil strata do not differ significantly from one another. It is to be emphasized, however, that this only represents culturable fungi, or those that are able to grow on synthetic media. This culture-dependent method of estimating fungal diversity may be a challenge especially when many fungi do not show diagnostic features or do not form fruiting bodies *in vitro* (Grube et al., 2017) and thus remain undetected for accounting and identification (Pozo et al., 2011).

In this study, the objective was to simply provide an overview of fungal richness or abundance and not absolute population data. This overview based on culturability is anchored on the fact that fungi are aerobic organisms and are thus dependent on oxygen for survival. An increasing soil depth decreases oxygen level (Noll et al., 2005) and is corollary to the results that CFU will also decrease. This trend is also shown to be true even using molecular techniques in bacteria (Noll et al., 2005) and arbuscular mycorrhizal fungi (Shukla et al., 2013).

Table 2. Mean[1] CFU of culturable fungi per mL of soil suspension ($x10^{-1}$) at different sampling depths of Lipa clay loam soil.

DEPTH (cm)	Number of morphotypes	Number of unique species	CFU/mL of soil suspension ($x10^{-1}$)
0-5 (surface)	24	20	90.75*a*
6-10 (sub-surface)	16	14	48.00*b*
11-20 (middle)	21	15	43.25*bc*
21-30 (deep)	8	8	17.75*c*

[1]*with four replicates*
Means followed by the same letter are not significantly different at p=0.0008 level of significance using Least Significant Difference Test

Bray-Curtis single link cluster analysis shows the similarity of taxa across the soil strata (Fig. 9). The surface layer had a 41.17%, 46.46% and 26.47% similarity with the subsurface, middle and deep layers, respectively. The subsurface layer had a 54.12% taxa similarity with the middle layer, while the middle layer recorded the least similarity with the deep layer at 23.53%.

Fungal Siderophore Identification

Consistent with literature, majority of fungi isolated in this study are able to produce hydroxamate siderophores (Table 4). Most Ascomycetes and Basidiomycetes synthesize the hydroxamate type of siderophores, classified into four main classes: ferrichromes, fusarinines, coprogens and rhodoturulic acid (Winkelmann, 1992). On the other hand, others produce the carboxylate type which contains citric-acid and is predominantly produced by members of the phylum Zygomycota (Das et al., 2007). Renshaw et al. (2002) reported the variety of these compounds across major groups of fungi. Unlike in bacteria where siderophores are strongly correlated to taxonomy, there is limited correlation that exists among fungi and the siderophores produced. Only the species *Aspergillus tamarii* was observed to produce all three types of siderophores. Six species, on the other hand, did not show any siderophore production. These were *Aspergillus terreus* Thom, *Fomitopsis meliae* (Underwood) Gilbertson, *Penicillium pinophilum*

Table 3. Species isolated across soil strata and corresponding frequency of occurrence in each layer of soil.

Species	surface	sub surface	middle	deep	Total FREQ.
	0-5 cm	6-10 cm	11-20 cm	21-30 cm	
Acrophialophora levis			1		1
Aspergillus aculeatus	1	3		1	5
Aspergillus brasillensis	10	3	2	1	16
Aspergillus flaviceps		4			4
Aspergillus flavus	3		4	1	8
Aspergillus fumigatus	3	2	5	1	11
Aspergillus tamarii				1	1
Aspergillus terreus	8	3	5		16
Aspergillus tubingensis	4	4	2	3	13
Aspergillus versicolor	2	1	1	1	5
Chaetomium globosum	4				4
Emericella nidulans		1			1
Emmia lacerata	1	3	1		5
Fomitopsis meliae			3		3
Fusarium sublunatum		3			3
Lentinus sp.	1				1
Mortierella sp.			3		3
Penicillium citrinum	2				2
Penicillium pinophilum	4	6	4		14
Penicillium polonicum	1			1	2
Penicillium solitum	3				3
Rigidoporus vinctus			2		2
Schizophyllum commune	1	5	2		8
Talaromyces verruculosus	3				3
Talaromyces pinophilus	2				2
Talaromyces radicus		2	2		4
Trametes maxima	1	4	4		9
Trichoderma erinaceum	3				3
Trichoderma harzianum	1		1		2
Total Frequency	**58**	**44**	**41**	**10**	**156**
Total Number of Species	**20**	**13**	**15**	**8**	**29**
Shannon Diversity Index	**1.19**	**1.10**	**1.12**	**0.86**	**1.07**

Hedgcock, *Talaromyces verruculosus* (=*Penicillium verrucolosum* B. Peyronel), *Trichoderma erinaceum* Bissette, C.P. Cubicek & Szacaks and *Trichoderma harzianum* Rifai.

The effect of environment on siderophore production has been reported in literature. A comparison of terrestrial and marine fungal isolates on their siderophore production revealed that although the nature of siderophores is independent of habitat, some fungi had more potent production than their counterparts from a different habitat. Terrestrial *Aspergillus niger* van Tieghem, for instance, had better production than its marine counterpart while marine *Aspergillus versicolor* (Vuillemin) Tiraboschi had more siderophores than its terrestrial counterpart (Baakza et al., 2004). Similar findings were reported by Vala et al. (2006) noting that difference in quantity of siderophores is a natural biological phenomenon. This points to an interesting search for fungal isolates from unique niches in a

Table 4. Siderophore production of fungal isolates.

Species	Phylum	Hydroxamate	Catecholate	Carboxylate
Aspergillus aculeatus	Ascomycota	+	-	-
Aspergillus brasillensis	Ascomycota	+	-	-
Aspergillus flaviceps	Ascomycota	+	-	-
Aspergillus flavus	Ascomycota	+	-	-
Aspergillus fumigatus	Ascomycota	+	-	-
Aspergillus tamarii	Ascomycota	+	+	+
Aspergillus terreus	Ascomycota	-	-	-
Aspergillus tubingensis	Ascomycota	+	-	-
Aspergillus versicolor	Ascomycota	+	+	-
Chaetomium globosum	Ascomycota	+	-	-
Emericella nidulans	Ascomycota	+	-	-
Emmia lacerata	Basidiomycota	+	-	-
Fomitopsis meliae	Basidiomycota	-	-	-
Fusarium sublunatum	Ascomycota	+	-	-
Lentinus sp.	Ascomycota	+	-	-
Mortierella sp.	Zygomycota	+	-	-
Penicillium citrinum	Ascomycota	+	-	-
Penicillium pinophilum	Ascomycota	-	-	-
Penicillium polonicum	Ascomycota	+	-	-
Penicillium solitum	Ascomycota	+	-	-
Rigidoporus vinctus	Basidiomycota	+	-	-
Schizophyllum commune	Basidiomycota	+	-	-
Talaromyces verruculosus	Ascomycota	-	-	-
Talaromyces pinophilus	Ascomycota	+	-	-
Talaromyces radicus	Ascomycota	+	-	-
Trametes maxima	Basidiomycota	+	-	-
Trichoderma erinaceum	Ascomycota	-	-	-
Trichoderma harzianum	Ascomycota	-	-	-

(+) positive/ producing; (-) negative/ not producing

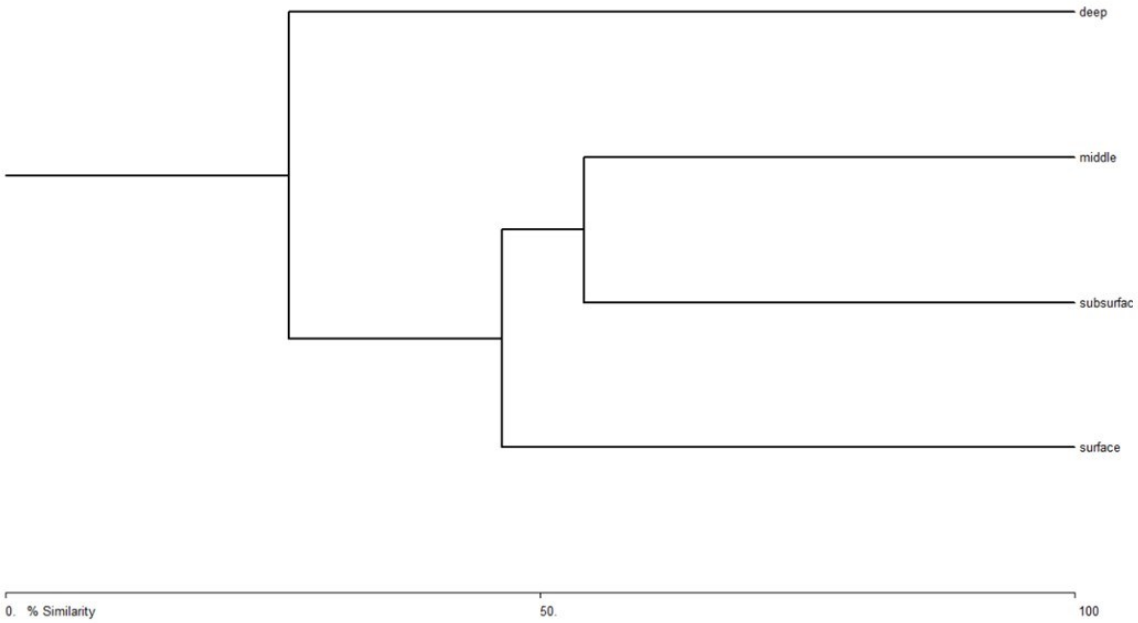

Figure 9. Results of the Bray-Curtis cluster analysis (single link) of soil strata with respect to fungal taxa.

comprehensive bioprospecting research.

Although siderophores are specific for iron, fungal siderophores are known to bind with other metals such as cadmium, lead, copper, magnesium, chromium, gallium, manganese and radionuclides at varying capacities (Nair et al., 2007) and thus is a potential mechanism for protection against heavy metal toxicity. *Fusarium solani* (Martius) Saccardo, for example, produces siderophores that contribute to the solubilization of copper and zinc *in vitro* (Hong et al., 2010).

The identification of fungi and the corresponding siderophores they produce can be useful especially in remediating metal-contaminated sites. Evidence available in literature highlights the central role of siderophores in the mobility of soil amendments. In the remediation of highly-contaminated soils, siderophores are extremely effective in solubilizing and increasing the mobility of a wide range of metals such as Cd, Cu, Ni, Pb, Zn, and actinides Th(IV), U(IV) and Pu(IV) (Ahmed and Holmström, 2014). The production of siderophores is site-specific, which means that unique ecosystems may trigger production of siderophores of fungal species as compared to their relatives from other habitats. Thus, exploring niches is essential as a means of bioprospecting for fungal species that can produce a wide range of siderophore compounds. As most soils are acidic, fungi have an advantage over their bacterial counterparts. Under pH conditions of 3-4, organisms that produce hydroxamate siderophores, mainly by fungi, have an advantage due to the extreme acid stability of these molecules.

Mycoremediation may address metal contamination concerns because of their intrinsic morphological and physiological characters, including their ability to produce siderophores. Because fungal species are versatile, they can be harnessed to withstand the dynamic soil environment as well as the metal pollutants present in it. The use of soil fungi, especially when in consortia, can be advantageous for higher metal scavenging and more stability against environmental fluctuations (Mishra and Malik, 2014) and can rely on the different mechanisms by which fungi detoxify metal contaminants (Pan et al., 2010).

Conclusion and Recommendation

This study is the first to comprehensively report the species of fungi in Lipa clay loam soil at UPLB. Twenty-nine species of fungi were isolated in this study. Most notably, hyphomycetes were the common isolates. A culture-dependent method resulted in low diversity of fungi. Nonetheless, species represented major groups of soil mycobiota, from hyphomycetes to coelomycetes of the phylum Ascomycota, to the Zygomycota and Basidiomycota groups. This current study showed at which soil

depth each species would most likely occur. Finally, results of siderophore identification were found to be in conformity with existing literature.

It is recommended that multiple isolation techniques be employed as well as direct soil DNA extraction to truly represent fungal diversity and eliminate the culture-dependent bottleneck. Siderotyping may be done to elucidate their functional role in responses of fungi to metal stress in actual metal exposure.

Acknowledgement

The authors acknowledge the funding by the Department of Science and Technology through the ASTHRDP Grant provided to the lead author. Researchers likewise acknowledge the assistance of the Soil Microbiology laboratory (Mr. Conrad Mamaril, Ms. Maria Czet Fulleros, Ms. Roselle Madayag, Mrs. Tess Q. Lantican, Ms. Shaira Granada, Mr. Patrick Cuenca, Prof. Lester Pide),Mr. Jeremy Roy Augustus Gomez (UPLB Plant Pathology) and Mr. Roy Boten (Bureau of Plant Industry) for the handling of specimens for identification.

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